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The Preparation and Some Properties of Crystalline Glucose 6-Phosphate Dehydrogenase from *Leuconostoc mesenteroides**

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ABSTRACT: Glucose 6-phosphate dehydrogenase has been purified from extracts of *Leuconostoc mesenteroides* by a simple procedure involving ammonium sulfate precipitation, treatment with protamine, ammonium sulfate fractionation, chromatography on hydroxylapatite, and crystallization. The enzyme is stable throughout the purification and in crystalline form. The enzyme exhibits dual nucleotide specificity and the ratio of activities with oxidized nicotinamide–adenine dinucleotide phosphate ($NADP^+$) and oxidized nicotinamide–adenine dinucleotide (NAD^+) remains constant throughout the purification. Both catalytic activities are similarly affected by various reagents and conditions. Reduced nicotinamide–adenine dinucleotide phosphate (NADPH) inhibits the NAD^+-linked reaction in a competitive manner. These results suggest that the same site on the enzyme binds both nicotinamide–adenine dinucleotides, and that no activating sites are present for either nucleotide. Neither dehydroepiandrosterone nor palmityl-CoA inhibits either of the catalytic activities of the enzyme. The properties of this enzyme are contrasted with those of the glucose 6-phosphate dehydrogenases from yeast and from lactating rat mammary glands.

Glucose 6-phosphate dehydrogenase was first isolated from *Leuconostoc mesenteroides* by DeMoss *et al.* (1953). The initial studies showed that this enzyme could utilize either NAD^+[1] or $NADP^+$ as hydrogen acceptor and that the ratio of the relative activities with these coenzymes remained constant throughout a purification of approximately 15-fold. DeMoss *et al.* also reported on the kinetic properties, pH optimum, substrate specificity, and inhibition of the enzyme. More recently, Kemp and Rose (1964) have shown that the NADH and NADPH produced by glucose 6-phosphate dehydrogenase in *L. mesenteroides* are reoxidized *via* distinctly different pathways. Thus, the NADPH provides the bulk of the hydrogens used for reductive biosyntheses, particularly of fatty acids, whereas NADH donates hydrogens to the products of fermentation.

Although glucose 6-phosphate dehydrogenases from a few other microorganisms display dual nucleotide specificity, notably the enzyme in *Acetobacter suboxydans* (Cheldelin, 1961), most glucose 6-phosphate dehydrogenases, like that in yeast (Warburg and Christian, 1936), cannot use NAD^+. A number of mammalian glucose 6-phosphate dehydrogenases, however, lack the strict specificity for $NADP^+$ which is characteristic of the enzyme from yeast (Levy, 1961). The enzyme from lactating rat mammary gland has been studied in detail in this regard. Under certain conditions, this enzyme reacts with NAD^+ at 7–10% of the relative rate with $NADP^+$. The K_m for NAD^+ is several orders of magnitude greater than that for $NADP^+$ (Levy, 1963). The NAD^+- and $NADP^+$-linked activities of the mammary enzyme respond differently to a variety of reagents and conditions, such as glycerol, NADPH, Mg^{2+}, $NaHCO_3$, orthophosphate, pH, phenanthridine, and dehydroepiandrosterone. These findings have been interpreted as suggesting the presence of two readily interconvertible forms of enzyme which exhibit different catalytic activities with NAD^+ and $NADP^+$; the equilibrium between these two forms was assumed to be affected by various of the reagents and conditions listed above (Levy *et al.*, 1966). It was further suggested that two distinct nucleotide binding sites were potentially available in this enzyme (Nevaldine and Levy, 1967).

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[1] Abbreviations used: NAD^+ and NADH, oxidized and reduced nicotinamide–adenine dinucleotide; $NADP^+$ and NADPH, oxidized and reduced nicotinamide–adenine dinucleotide phosphate.

In this paper we wish to report on the isolation and some properties of crystalline glucose 6-phosphate dehydrogenase from *L. mesenteroides*. This enzyme appears to be a single protein with a dual specificity for the nicotinamide–adenine dinucleotides. The same site on the enzyme apparently binds either $NADP^+$ or NAD^+. No evidence was found for an interconversion between two different forms of the enzyme.

Experimental Procedures

L. mesenteroides, ATCC 12291, was obtained from the American Type Culture Collection. The nicotinamide–adenine dinucleotides, glucose 6-phosphate, palmityl-CoA, and protamine sulfate (Salmine) were purchased from Sigma Chemical Co.; dehydroepiandrosterone from the California Corp. for Biochemical Research; and myristic acid from Eastman Organic Chemicals; Sephadex G-200, Blue Dextran 2000, and a 2.5 × 45 cm Laboratory column were obtained from Pharmacia Fine Chemicals, Inc. Yeast glucose 6-phosphate dehydrogenase was obtained from C. F. Boehringer and Sons. All other materials used were of reagent grade. Hydroxylapatite was prepared by the method of Levin (1962).

Ammonium sulfate, saturated at 4°, was neutralized with ammonium hydroxide and contained 1.0 mM EDTA. Buffer 1 contained 0.2 M NaCl, 1.0 mM EDTA, and 35 mM potassium phosphate, pH 7.2.

Enzyme assays were performed in a Zeiss PMQ II spectrophotometer at 25°. Assay mixtures contained the following components in a final volume of 3.0 ml: 33 mM Tris-HCl buffer, pH 7.8; 3.3 mM D-glucose 6-phosphate; and either 0.16 mM $NADP^+$ or 2.5 mM NAD^+ (neutralized to pH 7). Substrates and coenzymes were present routinely at a minimum of ten times the concentration of the determined K_m. Reactions were usually initiated either with enzyme or glucose 6-phosphate. In the experiments on NADPH inhibition and the effects of palmityl-CoA and fatty acids, it was advantageous to initiate with the appropriate nicotinamide–adenine nucleotide. The reaction was followed by noting the increase in absorbancy at 340 mμ with time. One unit is defined as the amount of enzyme required to catalyze the reduction of 1 μmole of $NADP^+$/min at 25°. K_m's for the reaction with $NADP^+$, or of glucose 6-phosphate in the presence of $NADP^+$, were determined by measuring $NADP^+$ reduction in a Farrand spectrofluorometer at 25° using an excitation wavelength of 350 mμ and a fluorescence emission wavelength of 460 mμ. Protein concentration was determined by the ratio of absorbancies at 280 and 260 mμ (Warburg and Christian, 1941) or, when levels of nucleic acid precluded use of this method, by a modification of the Biuret reaction (Gornall *et al.*, 1949). The apparent molecular weight of the enzyme was determined using a calibrated Sephadex G-200 column previously described by Nevaldine and Levy (1965).

Results

Isolation of the Enzyme. The pregrowth conditions and medium used were those described by DeMoss (1955). The cells were grown for 22 hr at 30° in five tightly stoppered carboys containing 10 l. of modified AC medium. They were collected by centrifugation in a Sharples Super centrifuge at a bowl speed of 45,000 rpm. The cell paste was resuspended in 0.1 M $NaHCO_3$ and the cells were washed in the same solution. The suspension was adjusted with 0.1 M $NaHCO_3$ so that a 1:100 dilution gave an OD_{660} of 0.4, and 50-ml volumes were sonicated for 40 min in a 20-kcycle Branson sonifier. The cellular debris was removed by centrifugation at 20,000*g* for 30 min. The supernatant was decanted and saved.

All subsequent operations were performed at approximately 4°, except where noted. The procedure for purifying the enzyme is given in detail below and the data for one preparation are summarized in Table I.

A saturated, neutralized solution of ammonium sulfate (1.5 volumes) was added to the crude supernatant. The precipitate was separated by centrifugation and discarded. Solid ammonium sulfate was added

TABLE I: Purification of Glucose 6-Phosphate Dehydrogenase from *L. mesenteroides*.

Step	Fraction	Vol. (ml)	Protein (mg)	Total Act. (units × 10^{-3})[a]	Sp Act. (units/mg of protein)[a]	Yield (%)
1	Sonicated cell supernatant	1030	11,700	27.4	2.33	100
2	60–100% $(NH_4)_2SO_4$ precipitate	71	1,460	24.7	16.9	90
3	Protamine supernatant	345	828	22.7	27.4	83
4	80–100% $(NH_4)_2SO_4$ fraction	24.8	234	20.8	93.9	76
5	Hydroxylapatite column eluate	83	45.9	9.76	220	36
6	1st crystals	0.75	22.9	6.10	266	22
7	2nd crystals	0.68	21.8	5.13	256[b]	18.6

[a] Activities refer to the activity with $NADP^+$ under the conditions given in the text. [b] After adsorption of bound $NADP^+$ with acid-washed charcoal, specific activity was 290 units/mg.

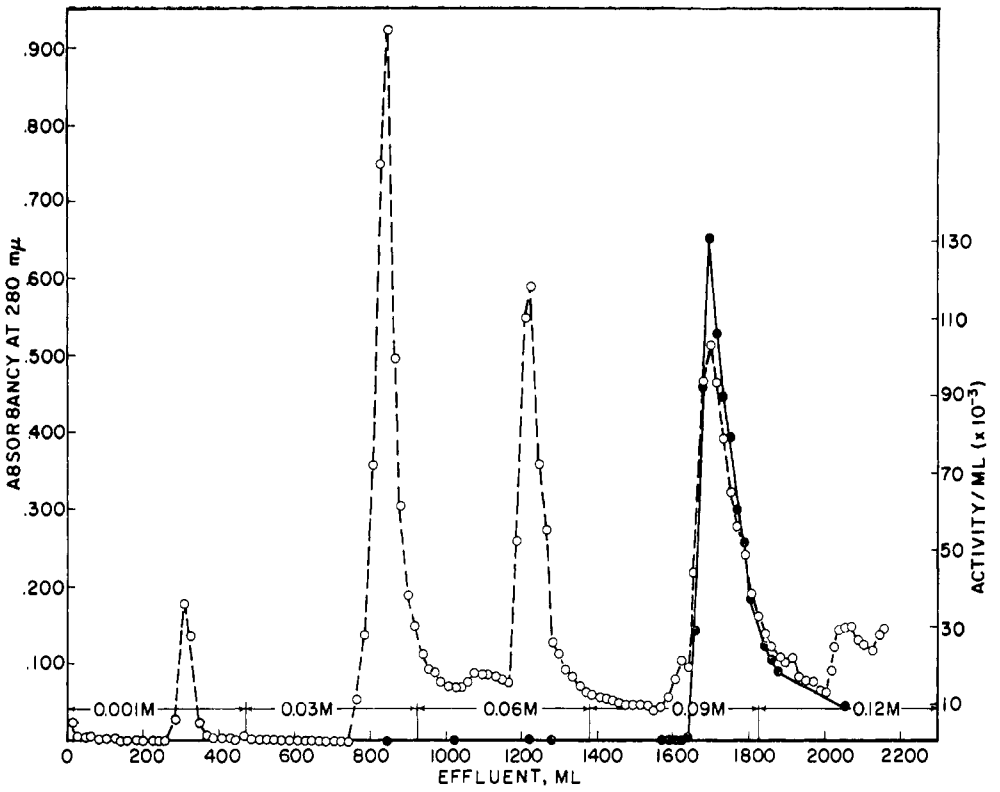

FIGURE 1: Elution pattern of protein and glucose 6-phosphate dehydrogenase from hydroxylapatite column. (—○—) Absorbance at 280 mμ; (—●—) units of enzyme per milliliter ($\times 10^{-3}$).

slowly to the supernatant until the solution was saturated. The solution was allowed to stir for 1 hr and the precipitate was collected by centrifugation. The precipitate was redissolved in a small volume of 0.01 M Tris-HCl, pH 7.8, containing 1 mM EDTA and the solution was dialyzed overnight (16–18 hr) against 100 volumes of the same buffer.

The dialyzed extract was diluted with twice-distilled water to a protein concentration of approximately 5 mg/ml, as determined by the Biuret method, and adjusted to pH 6.1 by the dropwise addition of 1.0 M acetic acid to the stirred solution. Protamine sulfate, 1.7%, pH 4.6–4.7, was added to the stirred solution and after removal of the precipitate by centrifugation, the supernatant was assayed for enzymatic activity; the concentration of nucleic acids was estimated from the absorption at 260 and 280 mμ (Warburg and Christian, 1941). When 92–95% of the nucleic acid had been removed, the pH of the supernatant was increased to pH 7.2 and any precipitate which formed was removed by centrifugation. The protein solution was then brought to 80% of saturation with solid ammonium sulfate. The precipitate was discarded and the supernatant was concentrated by precipitation at 100% ammonium sulfate saturation. The precipitate was dissolved in a minimum volume of 1.0 mM Na_2HPO_4–KH_2PO_4 buffer, pH 6.8, and dialyzed overnight against the same buffer.

The dialyzed solution was applied to a column (4 × 24 cm) of hydroxylapatite and was eluted with phosphate buffer at room temperature. Aliquots of buffer were used which were 1.5 times the column void volume and of increasing concentration, as suggested by Levin (1962). The enzyme is eluted in 0.09 M buffer (see Figure 1). The fractions containing enzyme of high specific activity were concentrated using Sephadex G-200 followed by precipitation at 100% ammonium sulfate saturation.

The resulting precipitate was dissolved in 0.5 ml of buffer 1, containing ammonium sulfate at 30% of saturation and 5×10^{-5} M $NADP^+$. The enzyme was crystallized by the dropwise addition of a saturated solution of ammonium sulfate (1 or 2 drops/day) over a period of a few days. Upon the appearance of the silky sheen which is characteristic of solutions of crystalline protein the further addition of ammonium sulfate was discontinued and the solution was allowed to stand for a few days while the turbidity of the crystalline suspension increased. The suspension was then centrifuged at 5000*g* for 60 min. The supernatant was withdrawn and the crystals were redissolved in the same buffer. The above procedure was repeated until a second crop of crystalline enzyme was obtained. The crystals appeared as very fine needles (see Figure 2).[2]

[2] We thank Mr. David Ellar of this Department for taking this photomicrograph.

TABLE II: Michaelis Constants of *L. mesenteroides* Glucose 6-Phosphate Dehydrogenase.[a]

Reaction	Substrate	Substrate Concn (M)	K_m for Substrate (M)
$NADP^+$ linked$^+$	$NADP^+$	$(8.3–170) \times 10^{-7}$	7.4×10^{-6}
$NADP^+$ linked$^+$	Glucose-6-P	$(1.7–100) \times 10^{-6}$	3.6×10^{-5}
NAD^+ linked$^+$	NAD^+	$(3.3–83) \times 10^{-5}$	1.15×10^{-4}
NAD^+ linked$^+$	Glucose-6-P	$(8.33–332) \times 10^{-6}$	6.4×10^{-5}

[a] The nonvaried substrate was present in every experiment at a concentration at least ten times its K_m. Michaelis constants were determined from Lineweaver–Burk plots. All experiments were performed in duplicate.

The enzyme has also been crystallized inside a dialysis bag by the slow addition of ammonium sulfate to the buffer in which the bag was suspended. These crystals were indistinguishable microscopically or catalytically from those prepared routinely as described above.

The enzyme has proved to be stable throughout its purification. A slightly purified preparation could be stored at −20° in 60% ammonium sulfate for nearly 2 months with a loss of about 7% of its initial activity. The crystalline preparation was stored for 1 month at 0–4° in 0.1 M Tris buffer at a protein concentration of 2 mg/ml with a loss of 15% of its enzymatic activity. This highly purified preparation is more stable when stored at higher protein concentrations.

A purification of 110–130-fold was generally obtained. The exact specific activity varies somewhat from preparation to preparation; the highest specific activity yet obtained is 315 units/mg, which may be compared with 67.5 units/mg of protein for crystalline protein from bovine udder (Julian *et al.*, 1961), 676 units/mg for the crystalline enzyme from yeast (Noltmann *et al.*, 1961), and 750 units/mg for the crystalline enzyme from erythrocytes (Yoshida, 1966). The erythrocyte enzyme is the most active glucose 6-phosphate dehydrogenase known. The specific activity of the *Leuconostoc* enzyme with NAD^+ as coenzyme approaches it in catalytic activity (see Table III).

Properties of the Enzyme. KINETIC CONSTANTS. The Michaelis constants for the enzyme with NAD^+, $NADP^+$, and glucose 6-phosphate are summarized in Table II. The K_m for NAD^+ is 16-fold higher than it is for $NADP^+$. The K_m for glucose 6-phosphate is nearly independent of the coenzyme involved. In contrast, with the mammary enzyme the K_m for NAD^+ is 1700 times greater than for $NADP^+$, and the K_m for glucose 6-phosphate differs by a factor of 16 depending upon the coenzyme used (Levy, 1963).

RATIO OF ACTIVITIES WITH NAD^+ AND $NADP^+$. *Leuconostoc* glucose 6-phosphate dehydrogenase appears to be a single enzyme which exhibits dual specificity toward the nicotinamide–adenine dinucleotide coenzymes. The NAD^+:$NADP^+$ activity ratio, followed over the course of the purification, remained constant at 1.8. Table III indicates the results that were obtained with various extracts at different stages of a purification. Both activities were assayed under conditions of substrate and coenzyme saturation.

EFFECTS OF VARIOUS REAGENTS AND CONDITIONS. Mammary glucose 6-phosphate dehydrogenase, like the *Leuconostoc* enzyme, displays a constant ratio of activities with $NADP^+$ and NAD^+ during its purification (Levy, 1963). Nevertheless, the two activities of the mammary enzyme are differentially affected by a variety of reagents and conditions, suggesting the presence of two different forms of the enzyme in facile equilibrium with one another (Levy *et al.*, 1966) and the existence of two distinct types of nicotinamide–adenine dinucleotide binding sites on the enzyme surface (Nevaldine and Levy, 1967). In order to ascertain whether an analogous situation occurs with the *Leuconostoc* enzyme, it was subjected to those reagents and conditions which had been found to affect the two mammary enzyme activities differentially.

At concentrations up to 32 mM, Mg^{2+} had a slight inhibitory effect (less than 20%) on the reactions with both NAD^+ and $NADP^+$ with the *Leuconostoc* enzyme. Bicarbonate produced a slight stimulation (less than 20%) of both activities when tested at concentrations up to 0.3 M. Increasing concentrations of glycerol produced parallel inhibitory effects on both activities; at a concentration of 30%, both activities were approximately 70% inhibited. Dehydroepiandrosterone, tested at concentrations of 10^{-4}, 10^{-5}, and 10^{-6} M, was with-

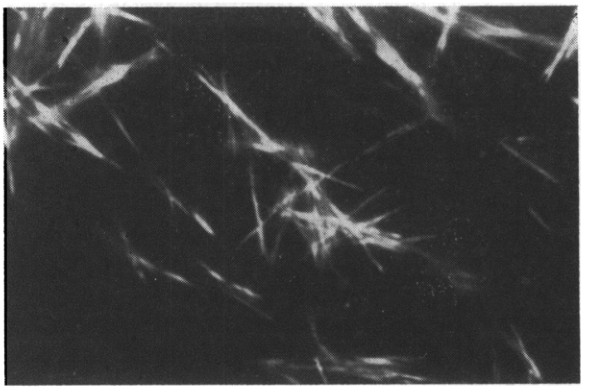

FIGURE 2: Crystalline glucose 6-phosphate dehydrogenase from *L. mesenteroides* (dark field, ×1250).

TABLE III: Reactivity of *Leuconostoc* Glucose 6-Phosphate Dehydrogenase with NAD^+ and $NADP^+$.

Fraction	Sp Act. (units/mg of protein)	Rate with NAD^+[a]	Rate with $NADP^+$[a]	$V_{NAD^+}:V_{NADP^+}$
60% $(NH_4)_2SO_4$ precipitate	0.089	0.039	0.022	1.77
Crude extract	2.39	0.123	0.067	1.83
Hydroxylapatite eluate	203	0.112	0.061	1.83
Crystalline enzyme	315	0.102	0.056	1.82

[a] Rates are given as ΔOD_{340} per minute, using standard assay conditions and 0.1 ml of enzyme in each assay.

TABLE IV: Comparison of the Effects of Various Reagents and Conditions on the Glucose 6-Phosphate Dehydrogenases from Yeast, Lactating Rat Mammary Gland, and *L. mesenteroides*.[a]

	Yeast[b]	*Leuconostoc*[d]		Mammary Gland[e]	
	$NADP^+$	$NADP^+$	NAD^+	NADP	NAD
Phosphate, low concentration	+	+	+	−	−
Phosphate, high concentration	−	0	−	−	−
Mg^{2+}	+	−	−	+	−
HCO_3^-	*	+	+	−	+
Glycerol	*	−	−	−	+
Dehydroepiandrosterone	0[c]	0	0	−	0
Palmityl-CoA	−[d]	0	0	−[d]	−[d]
pH optimum	8.5	>9.0	7.8	8.6	9.1
NADPH inhibition	Competitive	*	Competitive	Competitive	Complex

[a] + (stimulation); − (inhibition); 0 (no effect); * (not tested). [b] Glaser and Brown (1955) except as noted. [c] Marks and Banks (1960). [d] Present study. [e] Levy (1963) except as noted.

out effect on either the NAD^+-linked or the $NADP^+$-linked reaction. In contrast, the effects of these four reagents on the reactions with $NADP^+$ and NAD^+ with mammary glucose 6-phosphate dehydrogenase are qualitatively different (Levy, 1963).

As the pH was raised from 7.2 to 9.0 there was a gradual increase in the rate of the $NADP^+$-linked reaction with the *Leuconostoc* enzyme, amounting to 23% at pH 9.0. Qualitatively this resembles the effect seen with *NAD*$^+$ in the mammary enzyme, but the effect is quantitatively much smaller for the *Leuconostoc* enzyme. Similarly, there is a qualitative similarity between the effect of pH on the *NAD*$^+$-linked reaction with the *Leuconostoc* glucose 6-phosphate dehydrogenase and the *NADP*$^+$-linked reaction of the mammary enzyme, *i.e.*, virtually no difference in rate over the pH range from 7.2 to 9.0; again, the variations with the bacterial enzyme are even less (about 5%).

The effect of phosphate was tested at concentrations up to 0.3 M. The reactions with both $NADP^+$ and NAD^+ behaved similarly, showing slight stimulations at low concentrations (a maximum stimulation of 7 and 18%, respectively, for the reactions with NAD^+ and $NADP^+$ at 0.05 M phosphate), which were overcome as the phosphate concentration was increased. With the NAD^+-linked reaction, inhibition was observed with higher phosphate concentrations. With the mammary enzyme the stimulation at low phosphate concentration is not seen and both activities are inhibited.

DeMoss *et al.* (1953) reported that NADPH is a competitive inhibitor of NAD^+ in the NAD^+-linked reaction of the *Leuconostoc* enzyme. This was confirmed in our experiments, thus supporting the idea that both coenzymes are bound to the same site on the enzyme. With the mammary enzyme, it may be recalled, the inhibition of the NAD^+ reaction by NADPH is not competitive; rather, a sigmoid dependence of velocity on NAD^+ concentration is seen in aqueous solution (Levy, 1963; Levy *et al.*, 1966).

EFFECTS OF PALMITYL-COA. Among various enzymes

reported recently to be inhibited by long-chain acyl-CoA esters are the glucose 6-phosphate dehydrogenases from yeast (Eger-Neufeldt *et al.*, 1965) and liver (Taketa and Pogell, 1966). The latter authors suggested that palmityl-CoA inhibition might result from a conformational alteration of the susceptible enzymes, but no evidence for such a mechanism was presented. Experiments in our laboratory have indicated that when mammary glucose 6-phosphate dehydrogenase is incubated with palmityl-CoA, it undergoes irreversible dissociation into enzymatically inactive subunits with an apparent molecular weight of 61,700.[3]

When the glucose 6-phosphate dehydrogenase from *Leuconostoc* was incubated for 2–10 min with 10 μM palmityl-CoA there was no detectable loss of activity with either $NADP^+$ or NAD^+ as coenzyme. Incubation of yeast glucose 6-phosphate dehydrogenase with palmityl-CoA under the same conditions produced a loss of over 90% of the activity of this enzyme. Myristic acid (6.6×10^{-4} M) was also without effect on the enzyme from *Leuconostoc*. Table IV summarizes some of the findings with various reagents and conditions, and contrasts the properties of glucose 6-phosphate dehydrogenases from *Leuconostoc*, yeast, and mammary gland.

Apparent Molecular Weight. Two determinations of the apparent molecular weight of *Leuconostoc* glucose 6-phosphate dehydrogenase were made using gel filtration on Sephadex G-200. At initial protein concentrations of 5.1 and 21 mg/ml, the apparent molecular weight of the enzyme was 126,000 and 151,000 respectively. By the same method the apparent molecular weights of glucose 6-phosphate dehydrogenase from yeast (Andrews, 1965) and mammary gland (Nevaldine and Levy, 1965) were found to be 128,000 and 130,000, respectively.

Discussion

Glucose 6-phosphate dehydrogenase comprises approximately 0.5–1% of the total soluble protein in the crude extract of *L. mesenteroides*. The enzyme is quite stable, even at low protein concentrations, both during the purification and in the purest form that has been obtained.

The K_m values reported here agree fairly well with those reported previously, except that the K_m for NAD^+ was reported to be almost tenfold lower for the partially purified preparation (DeMoss *et al.*, 1953). It may be significant for the metabolism of *Leuconostoc* that the glucose 6-phosphate dehydrogenase displays a greater affinity for $NADP^+$ (assuming K_m and K_s are approximately equal), but a greater catalytic activity with NAD^+. NAD^+ is present in much higher concentrations than $NADP^+$ in *L. mesenteroides* (London and Knight, 1966).

It seems evident that the reactions with $NADP^+$ and NAD^+ are both catalyzed by a single enzyme and that both coenzymes are bound to the same site on the protein. First, the ratio of these activities remains constant throughout the purification. Second, no large differences in the effects of various reagents and conditions on the activities with NAD^+ and $NADP^+$ are seen. Finally, inhibition of the NAD^+-linked reaction by NADPH is strictly competitive, as would be expected if both coenzymes are bound to the same site of one protein. Glucose 6-phosphate dehydrogenase from the lactating rat mammary gland, like the *Leuconostoc* enzyme, is a single protein reacting with $NADP^+$ or NAD^+, but dual nucleotide specificity apparently involves two forms of the mammary enzyme, and more than one type of nucleotide binding site (Levy *et al.*, 1966; Nevaldine and Levy, 1967).

There is no inhibition of the *Leuconostoc* enzyme by dehydroepiandrosterone. Marks and Banks (1960) suggested that dehydroepiandrosterone and other inhibitory steroids may well be of importance in the control of mammalian glucose 6-phosphate dehydrogenases, since these steroids are produced only in highly differentiated tissues. Steroids do not inhibit glucose 6-phosphate dehydrogenases from cells which are not subject to steroid hormone control, such as spinach and yeast (Marks and Banks, 1960).

Palmityl-CoA has no inhibitory effect on either of the activities of *Leuconostoc* glucose 6-phosphate dehydrogenase. This is surprising since the glucose 6-phosphate dehydrogenases from yeast and from mammalian tissues are strongly inhibited by long-chain fatty acyl-CoA esters (Eger-Neufeldt *et al.*, 1965; Taketa and Pogell, 1966). A number of other enzymes show an inhibition by palmityl-CoA (Taketa and Pogell, 1966) and thus its effect may be exerted on some common aspect of the physical structure of these enzymes. One possibility, suggested by experiments with mammary glucose 6-phosphate dehydrogenase,[3] is that some of these enzymes may be dissociated into inactive subunits.

The glucose 6-phosphate dehydrogenases from yeast, lactating mammary gland, and *Leuconostoc* all catalyze the same reaction and have similar apparent molecular weights, as determined by gel filtration on Sephadex G-200. Beyond these basic similarities they have distinctly different characteristics with regard to stability, catalytic activity, coenzyme specificity, and response to various reagents and conditions. There is no evidence as yet for any physiological importance of the NAD^+-linked reaction catalyzed by the mammary enzyme; the $NADP^+$-linked reaction is closely linked with mammary fatty acid synthesis (McLean, 1958). In *Leuconostoc*, however, evidence exists that glucose 6-phosphate dehydrogenase plays a dual role *in vivo*, leading to the generation of either NADH or NADPH. The former participates in catabolic reactions, by donating hydrogens to the end products of fermentation, lactate and ethanol; the latter is used for the reductive biosynthesis of fatty acids and other products (Kemp and Rose, 1964). The presence of both $NADP^+$ and NADPH has been demonstrated (Kemp and Rose, 1964) and confirmed[4] in *L. mesenteroides*. Possible

[3] H. R. Levy, unpublished experiments.

mechanisms whereby the two catalytic activities of the glucose 6-phosphate dehydrogenase from *Leuconostoc* may be regulated are currently under investigation in our laboratory.

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[4] Experiments of Dr. G. M. deDrets of Montevideo, Uruguay (personal communication, Dr. B. L. Horecker).